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THE ACTION OF NEOARSPHENAMIN AND NEOSALVARSAN ON THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES

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In connection with certain experiments on the action of chemicals on phagocytosis, I found that solutions of neosalvarsan, 1 : 10,000, caused a well marked increase in the activity of leukocytes. I have made similar experiments with neoarsphenamin, to determine whether it would cause an increase in phagocytosis and what its action on the leukocytes might be.

Normal human serum and leukocytes were used in the following experiments. It is necessary that the organisms used do not clump, and strains of Streptococcus viridans from measles and influenza were chosen, which were not spontaneously phagocytable, yet not so virulent as not to be readily engulfed. The cocci were grown 24 hours either on moist goat blood agar and the growth washed off in salt solution, or they were grown in meat infusion broth. The suspensions to be satisfactory can be only slightly cloudy.

To test the action of the arsenic compound on phagocytosis equal parts of the compound in salt solution, serum, suspensions of washed leukocytes and of bacteria are incubated 25 minutes. Salt solution alone is used in the controls. Such mixtures are smeared on slides and stained with carbol thionin. At least 100 polymorphonuclear leukocytes are counted, and the number of cocci per leukocyte as well as the number of cells taking part in phagocytosis noted. The results of such tests are illustrated in table 1.

To determine whether the effects on the degree of phagocytosis shown in table 1 are on the leukocytes themselves, tests may be made as follows: Equal numbers of washed leukocytes are suspended in different dilutions of the arsenical compound for one hour at room temperature; the leukocytes are then washed twice with salt solution by centrifugation, equal parts of serum and bacteria added, and the whole incubated for 25 minutes, when smears are made and the results determined by studying 100 polymorphonuclear leukocytes (table 2).

Received for publication Jan. 27, 1922.

It appears that in vitro solutions of neoarsphenamin, below 1 : 1,000, as a rule, stopped or greatly reduced phagocytosis, while dilutions between 1 : 10,000 and 1 : 100,000 promoted phagocytosis, phagocytosis being increased on an average more than two times. Higher dilutions produced no effect. This increase in phagocytosis was found to be due to the action of the drug on the leukocytes, which were about twice as active in the diluted arsenical solution as in salt solution. These experiments were repeated six times with similar results, two different organisms being used, as a rule, in each experiment.

TABLE 1

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, HUMAN LEUKOCYTES, STREPTOCOCCI AND NEOARSPHENAMIN

Dilution of Neoarsphenamin	Average Number of Streptococci in 100 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1: 400	0.08	4
1: 1,000	1.6	44
1: 10,000	3.3	44
1: 100,000	4.7	56
1: 1,000,000	2.2	32
Salt solution	2.3	38

TABLE 2

PHAGOCYTOSIS IN MIXTURES OF HUMAN SERUM, STREPTOCOCCI, AND LEUKOCYTES, WHICH HAD BEEN SUSPENDED FOR ONE HOUR IN NEOARSPHENAMIN OR SALT SOLUTION AND THEN WASHED TWICE WITH SALT SOLUTION

Dilution of Neoarsphenamin	Average Number of Streptococci in 100 Leukocytes	Percentage of Leukocytes Taking Part in Phagocytosis
1: 1,000	0.16	8
1: 10,000	0.82	28
1: 100,000	0.76	28
1: 1,000,000	0.33	12
Salt solution	0.34	16

Next 7 rabbits, weighing about 1,800 grams, were injected intravenously with 1 c c of distilled water containing 0.007 gm. of neoarsphenamin, prepared as for human injection. Leukocyte counts were made before and every 15 minutes following the injections, for from 1 to 2 hours, or until the counts became normal. The opsonic and cytophagic (phagocytic activity of the leukocytes) indexes were determined, the opsonic determinations being made in the usual way, equal parts of serum, rabbit leukocytes and bacterial suspension being incubated 25 minutes.

In these experiments the phagocytic activity of the leukocytes was determined as follows: The leukocytes of the rabbits were collected in 2% sodium citrate solution, centrifugated and washed once in normal salt solution to remove all traces of serum. It is, of course, essential to use suspensions containing approximately the same number of polymorphonuclear leukocytes. Such suspensions may be obtained by counting the number of cells in the circulating blood, collecting the same amount of blood from each rabbit, and then equalizing according to count. The same serum was used to provide opsonin in the tests of the leukocytes. Equal parts of serum, equalized leukocytic suspensions and bacterial suspensions were mixed in bent capillary pipets and incubated 25 minutes when the mixtures were smeared on glass slides and stained. The phagocytic activity of the leukocytes, that is cytophagic index, was determined by comparing the number of bacteria taken up by the leukocytes before and after the injection of neoarsphenamin.

Five of the 7 rabbits injected with neoarsphenamin showed a decided increase in the number of leukocytes in from 15 to 30 minutes following the injection. This increase varied from 3,200 to 22,000, the average increase being 10,000. The leukocytosis was transitory rarely lasting 15 minutes. The leukocytes were from 2 to 6 times more actively phagocytic than normal at 15 minutes after the administration of neoarsphenamin. In the other 2 rabbits leukocytosis developed in 45 and 75 minutes, and in these animals an increased phagocytic activity of the leukocytes was present 30 and 45 minutes, respectively, after the injection. No changes in the opsonic index were observed, that is, there was no increase in opsonin. The number and activity of the leukocytes remained normal after the intravenous administration of 1 c c of salt solution and after 1 c c of salt solution containing 0.0002 gm. of mercuric cyanide.

According to Dinnick,[1] the intravenous administration of arsenical compounds is followed by an appreciable leukocytosis. He found that arsenic disappeared from the blood in a few hours and concludes that probably the therapeutic effect is only exerted during the short time the drug is circulating in the blood stream.

The fact that the leukocytes of most of the rabbits were more active during the height of the leukocytosis raised the question whether the presence of young leukocytes or the arsenic directly was responsible for this increased activity. To study this question further, two patients were examined before and after the injection of 0.6 gm. of neo-

[1] Lancet, 1919, 1, p. 1055

salvarsan. In neither case was there a leukocytosis, but in each instance 30 minutes after the injection, there was an increase in the phagocytic activity of the leukocytes, the index being 3.6 in one patient and 4.3 in the other. No increase of phagocytic activity could be made out 15 minutes later, that is 45 minutes after the injection of neosalvarsan. As the activity of the leukocytes and the leukocytosis did not always coincide in the rabbits, and as there was increased leukocytic activity in both patients, but without leukocytosis, the conclusion seems warranted that the neoarsphenamin or neosalvarsan was directly responsible for the increased phagocytic activity.

Various investigators have demonstrated that arsenical compounds increase antibody production. Boehncke[2] and Strubell[3] found that salvarsan stimulates opsonin production, and according to Strubell the increase in opsonin occurs from 2 to 8 hours after intravenous injection. An increase in other antibodies (agglutinins, hemolysins, precipitins) and also in complement, after the administration of arsenical compounds, has been observed by Aggazzi,[4] Friedberger and Maruda,[5] Lippmann,[6] Boehncke,[2] Weisbach[7] and Togama and Kolmer.[8] Kröcher,[9] Brekke,[10] and Reiter,[11] however, were unable to demonstrate any influence on the formation of antibodies by the injection of salvarsan into rabbits.

The results presented in this paper emphasize once more that to analyze thoroughly the effects of certain conditions or substances on phagocytosis, it is necessary to determine their action on the leukocytes as well as on the opsonin. Cross[12] concluded that because there was no reduction of the opsonic power of the serum in certain conditions there was no reduction in the phagocytic activity of the blood; but manifestly this is not a safe conclusion because it is not known that the phagocytic power or activity of the leukocytes, themselves the active agents of phagocytosis, remained unchanged.

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[2] Ztschr. f. Chemother., O, 1912, p. 136.

[3] Berl. klin. Wchnschr., 1912, 49, p. 1078.

[4] Ztschr. f. Immunitätsf., O, 1909, 1, p. 736.

[5] Therap. Monatsch., 1911, 25, p. 288.

[6] Deutsch. med. Wchnschr., 1911, 37, p. 1693.

[7] Ztschr. f. Immunitätsf., O, 1914, 21, p. 187.

[8] Jour. Immunol., 1918, 3, p. 301.

[9] Ztschr. f. Hyg. u. Infectionskrankh., 1914, 78, p. 321.

[10] Beretning for Gades, Path. Inst., 1912-3, p. 74.

[11] Ztschr. f. Immunitätsf., O, 1912, 15, p. 116.

[12] Bull. Johns Hopkins Hosp., 1921, 32, p. 350.

SUMMARY

In proper concentrations, neoarsphenamin and neosalvarsan may increase the phagocytic activity of leukocytes, both in vitro and in vivo. In vivo the stimulating effect is rapid and of short duration, occurring, as a rule, within 30 minutes after intravenous injection. Further study is necessary to determine what part, if any, this stimulus of the phagocytic activity of the leukocytes plays in the curative action of neoarsphenamin and allied products.